Ulster Med J 2008; **77 (1)** 65-76

Abstracts

10th Meeting of the Irish Society of Human Genetics, Monday 24th September 2007.

Postgraduate Centre, Belfast City Hospital, Belfast.

PROGRAMME:

10.00 – 11.00	Registration / Tea and coffee
11.00 – 11.01	Welcome
11.01 – 12.00	Spoken presentations Plenary I
12.00 – 13.00	**Keynote address:** "Sweet dreams: using genome wide association methods to find genes for diabetes and obesity" Mark McCarthy, University of Oxford
13.00 – 14.00	Lunch + poster viewing
14.00 – 15.30	Spoken presentations Plenary II
15.30 – 16.00	Tea / coffee & poster viewing
16.00 – 16.15	Business meeting
16.15 – 17.15	**Plenary address:** "Genomic Approaches to Brain Diseases". Guy Rouleau, McGill University
17.15 – 18.00	Wine Reception / Presentations / meeting close

SPOKEN PAPERS:

S1. A prospective study of referrals from the Irish Traveller community to the National Centre for Inherited Metabolic Disorders

AM Murphy, C Halling, SA Lynch, AA Monavari, S Harty, E Crushell, EP Treacy.

The National Centre for Inherited Metabolic Disorders (NCIMD) and National Centre for Medical Genetics (NCMG), Dublin.

Irish Travellers are a nomadic people in whom early marriage, frequent child bearing and consanguinity are cultural norms. They number 22,445, <0.5% of the Irish population, 9.6% of the 1465 patients listed at NCIMD on January 1st 2007 were Travellers. To date 21 different inherited metabolic disorders (IMDs) are reported. Our aim was to prospectively survey all referrals from this community .The study is part of a larger ongoing project to compile a database of "Irish Traveller" genetic disorders to ensure appropriate planning of services and provision of care.

All referrals between January 1st and June 30th 2007 were reviewed, those with a Traveller background identified and the following information sought; source, reason & age of referral, diagnosis & outcome.

Twenty eight (age range 1 day-16 yrs) of the 84 (33%) patients referred were Travellers. 15 new diagnoses of IMDs were established; of which 3 were genetic disorders not previously noted in this population (MSUD, X linked ALD and Hyperinsulinism). Six patients were diagnosed because of a family member with an IMD. Eight are under investigation for suspected metabolic disorder (mitochondrial). The remaining five are thought to have an undiagnosed autosomal recessive disorder because of the presence of multiple affected siblings. Apart from the patient with mucolipidosis, appropriate treatment was commenced once diagnosis was established with a good outcome to date.

This study highlights the huge disease burden imposed by the increased frequency of genetic disorders in consanguinous communities and provides useful epidemiological information for service planning for patients with IMDs with particular reference to the Irish Traveller community

S2. Counselling issues in a family with a presumed non-pathogenic mutation in TSC2.

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A diagnosis of Tuberous Sclerosis (TSC) can impact significantly on both the individual and the family. We report a family where the clinical presentation and genetic findings have presented difficulties in approaching management. Case 1 is an 8 year-old boy, who presented at age 8 with mild angiofibromata, but with no history of seizures or learning difficulty. MRI brain scan reported subcortical and periventricular hamartomas, and a small shagreen patch was found on clinical examination. He therefore fulfilled the diagnostic criteria for TSC. Mutation analysis identified a missense change in TSC2 – R261W – which was also found in the child's mother. No other mutations were found, and the mutation is felt to be a rare non-pathogenic variant.

S3. Disorders of cholesterol biosynthesis in Ireland

J Chukwu, C Halling, ATaha, SA Lynch, AA Monavari, EP Treacy, AM Murphy.

The National Centre for Inherited Metabolic Disorders (NCIMD) and The National Centre for Medical Genetics, OLCH, Crumlin Dublin 12. (NCMG)

Seven disorders of cholesterol biosynthesis are recognized of which Smith-Lemli- Opitz syndrome (SLOS) is the most common. These disorders are associated with major developmental malformations, unusual for metabolic diseases.

We reviewed the databases at the NCIMD and NCMG in order to identify all patients with inborn errors of cholesterol synthesis diagnosed in the 10 year period between June 1st 1997 and June 1st 2007. Clinical features (congenital malformations, behavioural

phenotype, growth and developmental profile), biochemical features (plasma sterol profile at diagnosis), genotype, ethnic background and treatment were noted.

Seven patients (age range 3-45yrs) attend the NCIMD making cholesterol pathway defects the 13th most common condition treated here. An additional 6 deceased patients were identified from the database at the NCMGs. All patients had SLOS. None of the other 6 disorders were identified.

The seven patients are being treated with cholesterol supplementation in the form of a powder or egg yolk. The dose of cholesterol supplementation is titrated by monitoring growth, cholesterol and 7 dehydro-cholesterol levels and adjusting levels accordingly. There has been subjective improvement in general well-being and behaviour. Anecdotally parental perception is that supplementation has a huge beneficial effect. Cholesterol biosynthetic disorders are rare disorders in the Irish population but important to recognize as they are partially treatable.

S4. Enzyme Replacement Therapy in Northern Ireland

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The first commercially available ERT was imiglucerase for the treatment of Gaucher disease. This was available in Europe from 1997. Since then ERT has become available for Fabry disease, mucopolysaccharidosis type I (Hurler, Hurler-Scheie and Scheie), mucopolysaccharidosis type II (Hunter) and Pompe disease. In Great Britain patients travel to one of the recognised NSCAG centres to receive their ERT. Patients in Northern Ireland have been receiving ERT for nearly 5 years – some initially as part of clinical trials.

Current patients on treatment:

Disease	Total patients	Adult/ children	Male/ female	Infusions
Fabry	8	8 / 0	7 / 1	Fortnightly
MPS I	4	1 / 3	1 / 3	Weekly
MPS II	2	1/1	2/0	Weekly
Pompe	Due to start			

In addition we have treated 4 children with MPS I (Hurler) with a finite course of ERT pre and post bone-marrow transplant. All patients have reported an improvement in their quality of life and clinical improvement has been confirmed by regular assessments and supporting data will be presented. Many of our patients are now on home infusions and this is working very well.

S5. Natural selection and disease susceptibility at the coagulation *F13B* locus

Anthony W Ryan[1,2,3], David Hughes[1], Thomas Ryan[2], Ross McManus[2] and Mark Stoneking[1].

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High levels of inter-population differentiation at the coagulation *F13B* locus may be interpreted as evidence of localised natural selection. However, a neutral explanation is also possible. We re-sequenced 4.6kb of the gene, encompassing all exons, splice junctions and 1.4kb of the promoter in African, European and Asian samples. This revealed three major lineages, which correspond to the common protein alleles and differ from each other at a non-synonymous substitution in exon 3 and a novel splice acceptor in intron K. There is evidence that these lineages are not functionally equivalent, a pre-requisite for the action of natural selection. Furthermore, our case-control analyses confirm that variability at this locus modifies susceptibility to myocardial infarct (OR = 1.88 [1.18 – 2.99], P = 0.0047). When our sequence data were combined with additional sequences from the *SeattleSNPs* database, Fu and Li's test for selection suggested a significant departure from neutral expectations (D* = -2.92556, P = 0.02). Patterns of extended haplotype homozygosity from HapMap populations also provide evidence of adaptation ($P < 0.05$). Thus, several independent lines of evidence suggest that the *F13B* locus has been subjected to localised natural selection during recent human evolution. Possible causes of this selection are discussed.

S6. An assessment of Ireland as a population for whole genome association studies

Colm Ó'Dúshláine, Ciara Dolan, Alice Stanton, David Croke, Reetta Kalviainen, Samuel Berkovic, Terry O'Brien, Sanjay Sisodiya, David Goldstein, Derek Morris, Norman Delanty, Gianpiero Cavalleri.

Trinity College Dublin, St James' Hospital, Dublin 8.

The transferability of HapMap SNPs to different populations is a significant factor determining the success of whole-genome association studies. We examined the extent to which the linkage disequilibrium of HapMap SNPs agreed with the same estimates for these SNPs within a number of populations. Comparisons were made for "test" populations of Caucasian individuals from Ireland, UK, Finland and Australia (4424 SNPs genotyped in 1178 individuals, covering 279 genes). Higher overall concordance was observed between HapMap CEPH individuals and Irish and UK populations (Spearman Rho 0.72, $p<0.0005$), the latter also exhibiting the highest level of similarity to each other from pairwise comparisons all our test populations (Spearman Rho 0.76, $p<0.0005$). Similar results were obtained when comparing haplotype diversity (Spearman Rho IRL=0.96, $p<0.0005$; UK=0.97, $p<0.0005$) and tag portability estimates (Spearman Rho IRL=0.55, $p=0.0004$; UK=0.58, $p=0.0002$). These findings have implications for researchers seeking to carry out fine mapping studies of disease susceptibility loci, particularly when these loci are identified from studies where candidate SNPs are derived from a HapMap reference. Specifically, our data shows that certain populations are in better agreement with HapMap than others and thus are likely to have more power in identifying disease susceptibility loci.

S7. The glutamatergic synapse protein HOMER2 is associated with schizophrenia in the Irish population

William P. Gilks[1], Emma Allott[1], Gary Donohoe[1], John L. Waddington[2], Michael Gill[1], Aiden P. Corvin[1], Derek W. Morris[1]

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Glutamatergic synapse dysfunction has been implicated in schizophrenia pathogenesis. To identify potential susceptibility

genes, we combined data from genome-wide linkage studies, the synaptic proteome and from keyword searches of genomic databases for glutamatergic genes. HOMER2 is located at chromosome 15q24, a region of significant linkage to schizophrenia. The HOMER2 protein forms a scaffold for post-synaptic glutamate receptors. To investigate its role in schizophrenia we selected HapMap-based tagging SNPs, integrating our own novel HapMap genotype data on five predicted functional SNPs into the SNP selection algorithm. We genotyped 12 tagging SNPs in our Irish sample of 375 cases and 812 controls. Single-marker association analysis showed disease association at rs869498, rs7174726 and rs12913501 (each SNP $p<0.05$, OR>1.3). These three SNPs are located in a 25kb region of intron 1 of the gene but are not in high linkage disequilibrium with each other ($r^2=0.02$). There was significant association of all two-marker haplotypes of the three SNPs, notably rs7174726-rs12913501 ($p<0.0005$). This 25kb region covers 4kb unique to higher primates strongly predicted to contain a transcription factor binding-site. HOMER2 is developmentally regulated, controlling synaptic plasticity and calcium homeostasis. This information, combined with our association results identifies HOMER2 as a putative susceptibility gene for schizophrenia.

S8. A linkage and association study of hip osteoarthritis.

D McGibbon, C Benson, G Meenagh, G Wright, M Doherty, A Hughes.

Queen's University Belfast.

Genetic and environmental risk factors affect risk of osteoarthritis (OA). Our study aims to identify susceptibility genes for hip OA.

A total of 426 Northern Ireland hip OA patients were genotyped using microsatellite markers and non-parametric linkage analysis carried out on affected sib-pairs. A peak LOD score of 1.64 (p=0.003) at 25cM on chromosome 19 indicated this region as potentially harbouring an osteoarthritis susceptibility gene. The best candidate gene in this region was *COL5A3*, which is flanked by genes *OLFM2* and *RDH8*. An association study using single nucleotide polymorphisms (SNPs) on unrelated Northern Ireland cases confirmed interest within this region with a significant p-value for SNP rs4804474 in *OLFM2* (p=0.016). This result was reproduced in a separate collection of 280 hip OA cases collected in Nottingham (p=0.03). The Northern Ireland and Nottingham cases combined gave the most significant p value of 0.009 for rs4804474. SNP's rs37455849(*COL5A3*) and rs889128(*RDH8*) give significant p-values in the Northern Ireland and Nottingham cases respectively of 0.037 and 0.025 but these values could not be reproduced. Further SNP genotyping across this region is required to fully elucidate the pattern of association and the location of the hip OA susceptibility gene.

S9. The Pathway to Breast Cancer Invasion.

Seona McErlean, Natalie Scott, Jenny Worthington, Gillian Brown, Adriana Falchi, Daniel Berrar, Anthony J Bjourson.

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Background: Considering the fact that the majority of breast cancer deaths are due to metastatic rather than the primary tumours, comparatively little consensus exists on the mechanisms of cancer invasion and spread to distant sites. In an attempt to better understand the invasive and metastatic processes we developed a model of breast cancer invasion and metastasis *in vitro* and subsequently validated this in a mouse model.

Methods: We developed a minimalist model of breast cancer invasion *in vitro* by converting a well known weakly-invasive breast cancer cell line into a series of progressively hyper-invasive sub-clones, ranging from non-invasive – to highly invasive. To identify the master regulators of invasion we performed micro-RNA (miRNA), messenger RNA (mRNA) transcriptional profiling and selected promoter methylation analyses on the non-invasive parental and selected hyper-invasive sub-clones. Systems biology pathway analyses was employed to identify genes that may represent key regulators of invasion. To validate the *in vitro* data, the parental (non-invasive) and hyper-invasive lines were stably transfected with a luciferase and their growth and metastatic spread was monitored in SCID mice using a Xenogen whole body imaging system.

Results: The pathway to invasion was clearly associated with an epithelial-mesenchymal transition (EMT), and a profound reduction in extracellular matrix (ECM) adhesion, altered cadherin expression, and silencing of interferon-γ (IFNγ) responsive genes consistent with archetypal immunoediting. Significantly however, this occurred independent of any Darwinian immune selective pressure and the simple process of selecting hyper-invasive cells concomitantly selected for a population that surprisingly, were also highly resistant to apoptosis (tolerant of hypoxia, more resistant to γ radiation, and more Trail-resistant). In addition, whole body imaging demonstrated that the *in vitro* selected cells were extremely invasive *in vivo* in SCID mice and rapidly metastasised to multiple organs within 3-4 weeks.

Conclusion: We have generated a useful model of invasion and metastasis. The genes identified appear to be directly related to the *primary cause*, rather than the *consequence* of cancer invasion & metastasis and may find utility as novel biomarkers and novel targets for therapeutic intervention.

S10. Sexual antagonism and autism susceptibility in the Xq/Yq pseudoautosomal region (PAR2)

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Autism is a neurobehavioural disorder associated with impaired language development, poor social interactions and stereotyped repetitive behaviours. We hypothesised that deregulation of the Xq/Yq pseudoautosomal region (PAR2) is involved in the profoundly male-biased affected sex ratio in autism. We therefore carried out TDT analysis on 95 autism multiplex families using 21 genetic markers in this region. In the proximal zone, which contains the brain-expressed imprinted *SPRY3* and *SYBL1* genes, we observed that multiple markers exhibited linkage / association with autism. In a further analysis involving datasets from which all male offspring or all female offspring were removed, we observed over-transmission of 'opposite' marker alleles to affected males and females for approximately half of the marker loci examined. We interpret these findings as evidence of sexually antagonistic selection operating at this locus. Our observations have general implications for human genetic studies and, more specifically, for the evolution and function of PAR2 genes.

POSTER PRESENTATIONS:

P1. EuroGentest: Quality Management and accreditation of genetic testing services

David E. Barton[1], Ros J. Hastings[2], Sarah Berwouts[3], Christine Brady[1], Philippe Corbisier[4], Anniek Corveleyn[3], Rob Elles[5], Brian Fowler[6], David Gancberg[4], Piotr Litynski[6], Milan Macek Jr[7], Ute Malburg[8], Gert Matthijs[3], Michael Morris[9], Clemens Mueller[8], Nick Nagels[3], Bettina Quellhorst-Pawley[2], Alexandra Stambergova[7], Jan Vermeesch[3], Kate Vickers[5], Elisabeth Dequeker[3].

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2 John Radcliffe Hospital, Oxford, United Kingdom,
3 KU Leuven, Leuven, Belgium,
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5 St. Mary's Hospital, Manchester, United Kingdom,
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7 Charles University, Prague, Czech Republic,
8 Universitaet Würzburg, Würzburg, Germany,
9 CMU, Geneva, Switzerland.

The EuroGentest network aims to improve and harmonize the quality of genetic services in Europe, from test development through to information for patients. The network encompasses Biochemical, Clinical, Cyto- and Molecular Genetics, Genetic Counselling and patient groups. Since January 2005, the EuroGentest Quality Management group has disseminated information on accreditation through five international workshops. A database on the current status of QAu in European genetic testing services will soon be publicly available. On the EuroGentest website, laboratories can find the EQA scheme most appropriate to their needs through discipline specific registers of schemes in Europe. All three laboratory disciplines have expanded their repertoire of EQA including a pilot pan-European cytogenetics scheme, CEQA. Minimum quality guidelines have been published for cytogenetics and some biochemical analytes. Draft guidelines for microarrays will be published later this year. In collaboration with EMQN, best practice meetings will be organised in 2007 for Familial Breast Cancer, Spinocerebellar Ataxias and Maturity Onset Diabetes of the Young to generate consensus guidelines. Finally QCMs for Prader-Willi/Angelman syndromes are being developed and validation of MLPA, diagnostic CF-testing kits and DNA extraction methods are in progress through a core group of accredited laboratories with reports due this year.

P2. Osteopetrosis: clinical and skeletal findings in 2 early childhood cases.

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Osteopetrosis is a rare, heterogeneous condition, characterised by osteoclast failure and classified into three forms: infantile malignant autosomal recessive osteopetrosis (ARO), intermediate autosomal recessive osteopetrosis (IRO) and autosomal dominant osteopetrosis (ADO). We present an unrelated 8 year-old girl and 5 year-old boy with a clinical and skeletal diagnosis of osteopetrosis and discuss the difficulties in determining recurrence risk in isolated cases.

Case 1. Elder of 2 sibs; non-consanguineous, clinically normal parents. Referred because of dental anomalies. Noted to have macrocephaly, short stature and prominent upper tibiae. No history of fractures. Skeletal survey showed findings consistent with IRO. No evidence of bone marrow compromise, abnormal renal function or cranial nerve compression.

Case 2. Youngest of 3 sibs; non-consanguineous, clinically normal parents. Fractures of both tibiae following trivial injuries age 2y. Normal stature with frontal bossing. Skeletal survey suggested type 1 ADO although IRO not out ruled. No evidence of bone marrow compromise or abnormal renal function.

Mutations in the ClCN7, ATP6i and other genes have been identified, but not all genes determining OP are known. In isolated cases of OP, diagnosis of type, and therefore recurrence risk, still relies on clinical and radiological findings. Some cases remain difficult to classify resulting in ambiguity over recurrence risks.

P3. The Genetic Basis Of Autosomal Recessive Osteogenesis Imperfecta In The Irish Traveller Population

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Osteogenesis Imperfecta (OI) is usually an autosomal dominant disorder, and is clinically classified according to the Sillence classification of 1-IV. However, the Irish Traveller population has an autosomal recessive form of severe OI, which fits with type II/III in the Sillence classification. We have identified 16 patients in 5 extended Traveller families, where almost all the affected children are born with severe limb and thoracic deformities due to multiple fractures, including *in utero* fractures. Most have died within 6 months, of respiratory compromise. However, there are two surviving affected children at ages 5 years.

No type I collagen abnormality has been described in the Irish Traveller OI (Pope *et al.* 1989). Recently, the genetic basis of one form of autosomal recessive OI has been found, due to homozygous mutations in a gene CRTAP or cartilage associated protein (Morello *et al.* 2006). CRTAP is homologous to a family of prolyl 3-hydroxylases which modify collagen, and mutations affected the modification of collagen fibrils. A partner protein for CTRAP, LEPRE1 or prolyl 3-hydroxylase 1 (P3H1) has also found to be the basis of another autosomal recessive form of OI in people of African origin (Cabral *et. al.* 2007).

Samples from 3 affected Irish Traveller children were analysed for mutations in CRTAP and P3H1. No mutations were found in CTRAP, but all three were homozygous for a frameshift mutation c.232delC in exon 1 of the P3H1 gene. Cultured fibroblasts from one affected case were analysed by mass spectroscopy for prolyl 3-hydroxylation of type I collagen. The level of hydroxylation was markedly reduced, at a level of 15%, compared to 95-98% seen in normal controls.

These findings have now identified the genetic basis for autosomal recessive OI in the Irish travellers. This will now lead to improved OI diagnosis and genetic counselling for Travellers who have a family history of severe or lethal OI. In addition, these findings give further insights into the biology of bone collagen.

P4. Investigation of the impact of the 19bp Deletion polymorphism in Intron 1 of Dihydrofolate Reductase (DHFR) on gene expression.

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Dihydrofolate reductase (DHFR) is an important folate metabolising enzyme that is essential to cellular proliferation because of its role in regenerating tetrahydrofolate (THF) from dihydrofolate (DHF), which is formed during the folate-linked synthesis of thymidine. Folate genes are considered candidates for association with neural tube defects (NTDs) such as spina bifida due to the preventative effect of periconceptional maternal supplementation with folic acid. Investigation of an intronic 19bp deletion polymorphism within the *DHFR* gene found a significant protective effect in mothers of NTD cases when present in one (Relative Risk 0.59 (95%CI: 0.39-0.89), p= 0.01) or two copies (Relative Risk 0.52 (95%CI: 0.32-0.86), p= 0.01). Analysis of mRNA levels revealed a small increase in expression (~1.5 fold) associated with the 19bp intronic deletion polymorphism, but this was not significant (Parle-McDermott *et al*., *Am J Med Genet* 2007;**143(11)**:1174-1180).

We sought to further investigate the potential impact of the DHFR 19bp intronic deletion polymorphism on gene expression by employing a recombinant dual luciferase system. PCR products representative of DHFR intron 1 with and without the 19bp deletion were cloned into a Gateway® compatible pGl_3- promoter vector and verified by sequencing. Luciferase assays will be performed in HEK293 cells and the data presented.

P5. Disease frequency of Inborn Errors of Metabolism in the Irish Traveller Community

AM Murphy.

The National Centre for Inherited Metabolic Disorders (NCIMD) and National Centre for Medical Genetics (NCMG), Dublin

The frequency of Inherited Metabolic Disorders (IEMs) varies between ethnic groups, reflecting founder effect, genetic isolation, and the potential effects of consanguinity. These disorders are a major cause of morbidity and mortality in "Irish Travellers", an endogamous group of nomads who number 22,000 in Ireland and 15.000 in the UK.

We aimed to compare the birth prevalence of IEMs in Traveller with non-Traveller children attending a tertiary level metabolic centre and to examine possible genetic factors contributing to observed differences. A retrospective review of diagnoses in Travellers was performed for 5 years (2002-2006). Mean birth prevalence was calculated and compared with overall figures for IEM's in the total population.

Travellers constitute 9% of the total patient group, but only 0.5% of the Irish population. 21 IEMs were noted, Galactosaemia, MPS I, Mitochondrial cytopathies, Glutaric Aciduria Type I,GSD Type IIIa, Mucolipidosis Type II, Hyperprolinemia Type 11,progressive familial intrahepatic cholestasis Type I (PFIC1) and carbonic anhydrase deficiency being the commonest. The birth prevalence of IEMs in the Traveller group for this period was 12/1000. That for the total population was 2.45/1000. Common homozygous mutations in all cases of galactosemia (Q188R), MPS 1(W402X), GA1(E365K), Mucolipidosis type II (c.3502_3delCT), Hyperprolinemia Type II (G521fs(+1)) and PKU (R408W) confirm the homozygous nature of this ethnic group.

We propose that the high incidence of IEMs in Irish Travellers may reflect initial founder effects and the increased rate of consanguinity.

P6. Myoclonus Dystonia- Phenotype- Genotype Correlation in the Irish Paediatric Population

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3. Department of Genetics, Teubingen, Germany

Myoclonus dystonia (DYT 11) is characterised by proximal myoclonic jerks and dystonia which causes torticollis and writers cramp. It has been associated with mutations on the epsilon sarcoglycan gene on chromosome 7q21 and may be alcohol responsive. An audit of over 70 patients seen at a quaternary paediatric movement disorder clinic revealed 21 patients with myoclonus dystonia.

Aim: to investigate clinical phenotype genotype correlation in Irish children with myoclonus dystonia.

Methods: 21 children in 17 M-D families were evaluated using a standardized neurological examination and review of video material. SGCE mutation analysis was performed on all patients.

Results: Age of onset ranged from 18 months to 14 years. A positive family history was seen in 11/21. Presenting symptoms were hand tremor, paroxysmal gait abnormality writing difficulties or a combination thereof. Clinical evaluation with pectoral muscles exposed showed irregular myoclonic jerks in all patients. SGCE mutation was found in 7/21 patients. All patients who had taken alcohol were alcohol responsive.

Conclusion: A typical adult M-D phenotype was rarely seen in children. Children were more likely to present with lower limb symptoms. Children with a positive mutation were more likely to present at an earlier age, were more likely to have a positive family history and were more likely to have lower limb symptoms.

P7. The IKBL protein inhibits TLR mediated activation of gene expression by NF kappa B

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The Inhibitor of NF Kappa B like *(IKBL/NFkBIL1)* gene encodes a protein homologous to members of the IKB family. *IKBL* is situated in the MHC and a number of different polymorphisms in the gene have been associated with diseases such as Myocardial Infarction, Rheumatoid Arthritis, Diabetes Mellitus, Celiac Disease and Crohns Disease.

The function of IKBL protein has not yet been reported. We have demonstrated, by both EMSA and Luciferase Assays, that over-expression of IKBL inhibits the activity of NF kappa B.

We have further demonstrated that IKBL inhibits NF kappa B activation by both TLR 2 and TLR 4 pathways. mRNA and protein expression of IL8, a NF kappa B regulated pro-inflammatory cytokine, was also inhibited by IKBL.

We show that IKBL and HDAC3 may co-localise in the nucleus offering a possible mechanism since HDAC3 is a known regulator of transcription factors.

Our study suggests that IKBL is a member of the novel inhibitors of NF kappa B such as MAIL that are located in the nucleus and may inhibit the activity of NF kappa B by regulating the activity of other proteins that bind to NF kappa B within the nucleus.

P8. Use of the Promega Powerplex® 16 kit to exclude maternal cell contamination in prenatal testing

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The exclusion of maternal cell contamination of prenatal samples is an important step in prenatal molecular genetic testing particularly when the prenatal sample shows the same genotype in the diagnostic test as the maternal sample. Traditionally, the exclusion of maternal cell contamination has been carried out by typing a number of linked or unlinked microsatellite markers from the maternal, paternal and foetal samples. This work becomes challenging when the microsatellite markers used are uninformative. If this occurs with several of the microsatellite markers selected for analysis the reporting of results may be delayed, as further microsatellite markers must be typed to complete the analysis. To assist in eliminating some of these problems, and with the additional aim of reducing the reporting time, we have been investigating the use of a commercial kit called Powerplex® 16 (Promega) for the exclusion of maternal cell contamination. This kit co-amplifies, in a single PCR reaction, 15 highly-informative microsatellite markers and the amelogenin locus. Here we describe the sensitivity of the Powerplex® 16 kit in detecting maternal cell contamination as low as 5% in prenatal samples.

P9. A systems approach to datamining association signals from whole-genome association studies

Colm Ó'Dúshláine, Derek Morris, Elaine Kenny, Carlos Pinto, Michael Gill, Aiden Corvin.

Trinity College Dublin, St James' Hospital, Dublin 8.

Whole-Genome Association (WGA) studies have the potential and power to detect a large number of significant associations with a disease phenotype. Understanding these findings can be difficult and replication is often an integral part of this type of study, but even this may only identify low hanging fruit. Published WGA data shows that, while novel genes have been found for the various phenotypes, a large number of genes still remain to be identified. For example, in a study of type 2 diabetes that found 5 genes for the disorder, Saxena *et al* (2007) commented that these 5 loci contribute only modestly to the overall variance in diabetes risk (~2.3%), indicating that many more genes remain to be found. We present a systems biology approach to mining WGA data. By availing of gene interaction data from KEGG HPRD BIND and Reactome, we integrate genes containing significantly associated SNPs into gene interaction networks. This provides a platform for inferring biological pathways enriched for disease associated genes. Our approach is flexible, taking account of the available interaction data and the number of significantly associated genes under investigation, and permits varying levels of intermediate interacting genes between the associated genes. We apply this method to the investigation of a number of existing WGA studies, highlighting heretofore unobserved pathway signals.

P10. Development of a *C.elegans* model system for genetic and molecular dissection of epigenetic mechanisms underlying trinucleotide expansion disorders.

Katherine Schouest[1], Avril Coghlan[2], Charles Spillane[1].

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Model organisms are essential for the rapid advancement of genetic research on human diseases. *C. elegans* has already proven to be a powerful biomedical model in dissecting principles underlying diseases like Huntington's Disease and Myotonic Dystrophy, as well as in revealing potential targets for therapeutic treatment. We are using *C. elegans* as a model organism to investigate epigenetic phenomena associated with nucleotide repeat disorders and to identify modifiers that regulate the size and rate of the expansions. To identify candidate tester loci for our research we have screened the *C. elegans* genome for trinucleotide repeats that have at least 12 trinucleotide repeats and a high level of purity, as these are most likely to undergo expansion or contraction. We have identified 20 such repeats located in *C. elegans* exons and 17 repeats located within introns. To select one or more variable repeats for further analysis, the extent of polymorphism of these repeats is currently being examined over 48 wild isolates of *C. elegans*. The identification of polymorphic repeat loci will allow monitoring of these alleles through multiple generations in control animals and animals depleted of candidate epigenetic modifiers via RNAi. The project will ultimately facilitate the understanding of the epigenetic phenomena causing nucleotide expansion disorders.

P11. Analysis of SOD1 gene for IVS 2+50 del 7 genomic deletion in Northern Irish Keratoconus Patients

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Purpose: Oxidative stress has been suspected as a major contributor to pathogenesis of keratoconus (KC) as accumulation of cytotoxic by-products from nitric oxide and lipid peroxidation, abnormal antioxidant enzymes found in KC cornea. Recently a heterozygous 7bp deletion in intron 2 (IVS 2+50 del 7) close to the 5' splice junction of the super oxide dismutase 1 (SOD1, MIM:147450) gene was reported in three KC patients (Udar *et al.,* IOVS; 2006). The purpose of this study was to screen for the IVS 2+50 del 7 of SOD1 gene in KC patients from Northern Ireland.

Methods: Blood samples were collected from 17 KC patients at RVH Eye Clinic and DNA was extracted from leucocytes. Conventional and FAM-labelled oligonucleotide primers were designed flanking the genomic deletion, IVS 2+50 del 7 of SOD1 gene. Screening for the intronic deletion was performed by PCR based direct cycle sequencing and fragment length analysis using ABI 3100 automated DNA sequencer.

Results: Seven out of 17 sporadic KC patients appeared to show a 2-3 bp genomic deletion within the previously published intronic region when sequencing in one direction, but there was no frameshift in the downstream sequence. Sequencing in the reverse direction and fragment length analysis failed to demonstrate any intronic deletion.

Conclusions: The genomic deletion (IVS 2+50 del 7) of SOD1 gene was not found in a subset of the NI population with KC. Sequencing results in this region should be interpreted with caution. Patient recruitment is ongoing and further analysis of the entire coding region of SOD1 is required to elucidate the role of this anti-oxidant

enzyme in KC. [Funding Authority: Research & Development Office, Northern Ireland].

P12. Predictive Testing for Carney Complex in a Child

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This is a case of a 4 year old girl with a three-generation family history of Carney Complex. Carney Complex is an autosomal dominant condition, with around 400 patients reported worldwide. The phenotype is variable and there may be a degree of under-diagnosis. Manifestations include skin pigment abnormalities, myxomas, endocrine tumours and schwannomas. The family mutation has been found, delta FSC18 PRKAR1A. All affected family members have characteristic facial freckling. The child had a milder distribution of facial freckles and was considered to be affected by the family. Her mother requested predictive testing for confirmation. No intervention is recommended routinely until puberty in Carney Complex, although cardiac myxomas may present at any time from birth. These can cause embolic events or obstruct blood flow, leading to sudden death. Our case raises the question of whether to test an asymptomatic child who has similar facial features as affected family members. In this situation, the child may consider themselves affected. This child tested positive for the family mutation. Her mother was brought back to the clinic alone to be informed. The child has been referred to a paediatric cardiologist. She will be offered a genetics consultation when she is older.

P13. Association studies of SEMA6A, SEMA6B, PLXNA2 and PLXNA4 genes in an Irish schizophrenia case-control sample.

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Schizophrenia has a substantial genetic component. Finding genetic variants that alter risk may help in identifying pathways that are aetiologically important, both in predicting illness risk and developing treatments. Work by our group and collaborators have identified the Semaphorin 6 and Plexin A gene families as putative susceptibility genes for schizophrenia. Here we report analyses of SEMA6A, PLXNA2, SEMA6B and PLXNA4.

SNP maps were generated for all four genes. SNPs located within the gene region were ranked according to their location. Priority was given to SNPs in coding regions, UTR's, splice junctions, promoter regions, evolutionary conserved regions, regions containing clusters of transcription factor binding sites (TFBS) and conserved TFBS. Tag SNPs were chosen using HapMap CEU linkage disequilibrium data. Altogether 74 SNPs were genotyped across the four loci in a sample of 375 schizophrenia cases and 812 controls.

Three SNPs at SEMA6A, two SNPs at SEMA6B and three SNPs at PLXNA2 reached nominal levels of significance (p=0.01-0.05) prior to correction for multiple testing.

Given the limited power of our association sample and the number of SNPs tested, these findings require independent replication. However, the results may point to a role for abnormal axon guidance and cell migration in schizophrenia pathophysiology.

P14. Absence of linkage to known loci in an Irish RLS family

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Restless legs syndrome (RLS) is a neurological sleep disorder characterised by abnormal sensations in the legs associated with an irresistible urge to move. Symptoms occur predominantly at rest and worsen at night, resulting in nocturnal insomnia and chronic sleep deprivation.

RLS has an estimated prevalence of 5 to 15% in the general population. Familial aggregation has been widely reported and the condition is predominantly an autosomal dominant disorder. Molecular genetic approaches have identified five loci on chromosomes 12q, 14q, 9p, 2q, and 20p, in RLS-affected families from different populations. No disease-causing gene has yet been identified. The increase in symptom intensity at night has implicated the circadian system and response to treatment with dopamine agonists has suggested an abnormality in the dopaminergic pathway.

An Irish family with autosomal dominant RLS has been recruited, with the aim to localise and identify the gene responsible for the syndrome. The five described RLS loci were examined for linkage; however results indicate that the new Irish RLS pedigree is not linked to the currently described genetic loci. This provides further evidence of genetic heterogeneity in RLS. Future work includes a genome-wide scan to identify the novel locus in this Irish RLS family.

P15. The *CHEK2*1100delC* variant: present in the west of Ireland breast cancer population.

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Background: As part of a population-based approach to breast cancer genetics, a West of Ireland cohort is under study to elucidate inherited variation which predisposes women to developing breast cancer. *CHEK2* has been identified as a low-penetrance breast cancer susceptibility gene conferring a 2-fold elevated risk of breast cancer in women and 10-fold in men.

Materials and Methods: To evaluate the prevalence of the *CHEK2*1100delC* variant, DNA collected from 591 breast cancer cases and 572 healthy controls were analysed. FAM (carboxyfluorescein) labelled PCR products were capillary separated on the ABI 3700 and fragment analysis carried out using Genotyper v2.5. Normal PCR fragments measures 168bp and the *CHEK2*1100delC* could be clearly seen at 167bp. A sequenced control *CHEK2*1100delC* patient DNA was PCR amplified for each test reaction.

Results: The *CHEK2 *1100delC* mutation was found in three cases, one had a sibling who was affected with colorectal cancer. The mutation was not found in any control samples.

Discussion: We have established that the *CHEK2*1100 delC* variant is present in the Irish population and is in excess in cases over controls. Our data are consistent with effect on risk. Its role in the clinical setting has yet to be elucidated.

P16. Genetic interaction assessment of major Age Related Macular Degeneration (AMD) susceptibility loci within the Northern Ireland population.

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Purpose: To assess the effect of Complement factor H (*CFH*), Factor B (*FB*), Component C2 (*C2*), *LOC387715/HTRA1* locus and the influence of Vascular Endothelial Growth Factor (*VEGF*) and Apolipoprotein E (*APOE*) within a Northern Ireland AMD cohort.

Methods: DNA samples (n=250) with end-stage wet AMD and an age and sex-matched control cohort (n=250) underwent ophthalmic examination with detailed medical and smoking history. Haplotype analysis was undertaken for *CFH*, *CFHR1*, *CFHR3*, *LOC387715*, *FB*, *C2*, *VEGF* and *APOE*. Haplotypic structure for each gene was determined from HapMap and tagged SNPs were multiplexed using SNaPshot technology (ABI).

Results: Results show a higher incidence of AMD risk haplotypes within the affected cohort with a decreasing incidence of protective haplotypes when compared to the controls for *CFH*, *C2/FB* and *LOC387715*. Genetic variation within *C2* and *FB* would appear to be less strongly associated with the disease cohort than previously reported. A significant role for *VEGF* in relation to wet AMD has not been shown.

Conclusions: It would appear that *CFH* and *LOC387715* remain the most strongly associated genetic factors with AMD and that *VEGF* is unlikely to have any significant involvement with disease manifestation within this population.

P17. C-banding analysis of a newborn with clinical features of Roberts syndrome

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Roberts syndrome (pseudothalidomide syndrome) is a rare autosomal recessive syndrome which presents with craniofacial abnormalities, limb, heart and renal defects. The condition has recently been mapped to 8p12, with mutations found in ESCO2, a gene essential for the establishment of sister chromatid cohesion responsible for clinical presentation (Vega *et al.,* 2005). A majority of affected individuals (about 80%) exhibit a chromosomal phenomenon known as "heterochromatin repulsion" (also referred to as premature centromere separation).

A newborn female infant of Polish origin was referred for cytogenetic investigation; her clinical features included multiple congenital anomalies, - hypertelorism, midline cleft lip and palate, severe symmetrical intra uterine growth retardation, absent radii and talipes.

Chromosome analysis revealed a female karyotype of 46,XX chromosomes. Approximately 50% of the cells examined exhibited a characteristic morphology with lack of a defined centromeric constriction with some pericentromeric regions being splayed out and appearing as "puffing". This phenomenon is characteristic of Roberts syndrome.

Limb defects, cleft lip and palate, multiple congenital anomalies; together with the characteristic heterochromatic repulsion are diagnostic of Roberts syndrome in this patient.

P18. Tuberous Sclerosis Complex – an audit of referrals to the Northern Ireland Genetic Service over a four period.

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In the four years 1 June 2003 –1 June 2007, 19 patients were referred to the Northern Ireland Genetic Service for investigation of Tuberous Sclerosis Complex (TSC).

Results: 13 patients have a clinically confirmed diagnosis of TSC. Of these, 7 patients had seizures age <12 months as a first presentation of TSC. In these patients, 2 undiagnosed parents were identified as being TSC affected following the diagnosis in their child. 3 patients (age range 9 – 17) presented with angiofibromata and a diagnosis of TSC was made following referral by the Dermatologist. 1 TSC affected adult had a previously confirmed diagnosis of TSC. This patient came to Northern Ireland from Portugal.

Conclusion: In a stable population, the majority of patients with TSC are diagnosed in infancy or early childhood. A second group of previously undiagnosed, mildly affected adults was identified, following the diagnosis of TSC in their child. A third group of older children and adolescents was identified. An emerging trend is seen, where a proportion of new referrals for TSC are patients who have come to Northern Ireland from other parts of Europe.

P19. Audit Of Myelodysplastic Syndrome Cases Submitted For Cytogenetic Analysis Over A Two Year Period.

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Background: Myelodysplastic Syndromes (MDS) are a group of conditions of progressive bone marrow failure of normal maturation leading to peripheral cytopenias. More than one cell lineage is involved, with often up to three. It is typically a disease of the elderly. There is a 20-40% risk of transformation from MDS to Acute Myeloid Leukaemia (AML). Chromosome abnormalities would be expected in 40-50% of MDS cases. Evidence suggests that cytogenetic analysis can influence the clinical evaluation. The clinical boundaries between MDS and AML are indistinct and a similar overlap occurs cytogenetically. Cytogenetic analysis, therefore, does not prove informative for a differential diagnosis.

Aim: To minimise the number of borderline MDS cases submitted for cytogenetic analysis with a view to improve efficiency. This should allow the department to broaden the range of tests offered.

Results: 805 MDS samples were received over a two year period ranging from age 30-90. 164 of these samples were deemed not required on review of Bone Marrow morphology. Of the remaining 641 samples 83 of these samples had an abnormal cytogenetic karyotype. This gives a 12.9% abnormality rate for MDS cases referred to this facility. 80% of the abnormal karyotype cases were aged between 60 and 90.

Discussion: As the expected abnormality range in a cytogenetic

laboratory for MDS is 40-50% it is clear that a more stringent selection criteria needs to be implemented. It is also evident from the high percentage of abnormal karyotype cases in the age range 60-90 that MDS is a disease of the elderly. It is proposed that cytogenetic analysis for all MDS cases should only be completed on receipt of a diagnostic bone marrow morphology report, a complete clinical history or by personal communication with the requesting consultant.

P20. Detection of subtelomeric rearrangements in children with unexplained mental retardation using Multiplex Ligation-dependent Probe Amplification.

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Mental retardation is a common lifelong disability affecting 1-3% of the general population. The cause of the disorder remains unknown in approximately half of all cases which can be a source of anxiety for the family of the affected patient. It has been reported that submicroscopic subtelomeric rearrangements may be responsible for 2.5-10% of all unexplained mental retardation cases.

Multiplex Ligation-dependent Probe Amplification (MLPA) can be used to detect cryptic subtelomeric imbalances. It has the advantage of being a cost effective, rapid and easy to use technique. We will present the results of 200 retrospective DNA samples tested for the presence of subtelomeric rearrangements using two different MLPA probe mixes. These samples are from children with unexplained mental retardation/developmental delay and normal karyotype and Fragile X results.

The objectives are to assess whether the introduction of a service in our laboratory to screen patients with idiopathic mental retardation/developmental delay for subtelomeric aberrations is practicable and of benefit to the Irish population and to determine whether MLPA is a suitable technique for the detection of these anomalies.

P21. There is no evidence of linkage or association between Parathyroid Hormone Receptor Type 1 polymorphisms or haplotypes with low bone mineral density in a Caucasian cohort

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Previous studies have observed suggestive evidence of linkage between low bone mineral density (BMD) and the parathyroid hormone receptor type 1 (PTHR1) locus (3p22-21.1). In the present study, we tested for association between genetic variants in the PTHR1 gene and variation in BMD.

Four single nucleotide polymorphisms (SNPs), located throughout the PTHR1 gene, were tested for association with BMD in 278 nuclear families and 500 unrelated postmenopausal Caucasian women.

There was no evidence of linkage between the PTHR1 genotypes and BMD using Merlin (LOD scores < 1.0). The Family Based Association Test (FBAT) was used to test for association between the PTHR1 genotypes and haplotypes with BMD. There was significant association between SNP rs4683301 with variation in BMD at the femoral neck (P = 0.03) and lumbar spine (P = 0.02) using the genotype model in FBAT. However, following adjustment for covariates, there was no significant association (P >0.05). The PTHR1 haplotype, h3 (TC), was significantly associated with BMD at both skeletal sites (P < 0.00). However, after correction for covariates, there was no significant association.

Denser SNP genotyping may be necessary to better define the possible relationship between the PTHR1 gene and BMD variation in this cohort.

P22. Association of Methylenetetrahydrofolate reductase (MTHFR) polymorphism and the risk of Squamous Cell Carcinoma in renal transplant patients.

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Background: The relative risk of developing cutaneous squamous cell carcinoma (SCC) is significantly increased following organ transplantation.

Objective: We investigated genetic association with SCC in two pathways associated with cancer risks, with potential for modification by vitamin supplementation.

Methods: 367 renal transplant recipients (117 with SCC and 250 without any skin cancer) were genotyped for key polymorphisms in the folate pathway (MTHFR: C677T; methylene tetrahydrofolate reductase), and the vitamin D pathway (VDR: Intron 8 G/T; vitamin D receptor).

Results: Individuals carrying the MTHFR 677T allele had a marked increase in risk of SCC (adjusted OR= 2.54, p=0.002, after adjustment for age, sex, skin type, sun exposure score and immunosuppression duration; lower 95% confidence boundary OR of 1.41). In contrast, VDR polymorphisms were not significantly associated.

Conclusion: Folate-sensitive pathways may play a critical role in the elevated rate of SCC in renal transplant recipients.

P23. Study of the Knowledge of Inherited Metabolic Disorders among patients and their families in the Irish population

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Galactosaemia and Maple Syrup Urine Disease (MSUD) are recessively inherited conditions screened for by newborn screening in Ireland. Affected patients are followed at the National Centre for Inherited Metabolic Disease. We aimed to assess the degree of genetic knowledge imparted to families to determine if further formal genetic counselling would be beneficial. Adult patients and parents of affected children were interviewed in person using a questionnaire including 4 demographic, 8 knowledge, 2 information and 5 impact questions. To date, 8 adults (7 galactosaemia, 1 MSUD) and 18 parents (12 galactosaemia and 6 MSUD) have been interviewed. All parents of children with MSUD correctly answered questions on MSUD and recurrence, but did not know the risk or implications of carrier status. For galactosaemia; 9 of 12 parents scored 6/8 or better on knowledge, while all adults scored 3 to 5 of 8. 16/26 study participants requested more information about their condition and its transmission. Affected adults also identified a need to meet others with the same condition. Our study to date indicates that parents of children with these genetic disorders are well informed, however adult patients could benefit from further genetic counselling. This may reflect a reluctance to transmit genetic information within families.

P24. Determination of the most stable endogenous control gene using an *in vitro* model of folate deficiency.

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Folate is an essential nutrient necessary for DNA synthesis, cellular proliferation and biological methylation reactions. Suboptimal folate status is a risk factor for several human diseases. An understanding of the molecular mechanisms linking folate status to these conditions is still incomplete. In a bid to dissect the molecular response to folate status we set up an *in vitro* model of folate depletion. RT-PCR is currently the method of choice to examine expression levels of a specific set of genes. Key to this method is normalisation of results to an appropriate endogenous control gene that is relatively unaffected by the experimental conditions. Inaccurate normalization can lead to findings that do not reflect the true experimental variation. We sought to identify the most appropriate endogenous control gene for normalisation of gene expression data in our *in vitro* model of folate depletion in HEK293 cells to ensure only true gene-specific variation in response to folate levels will be reported. This was undertaken using the TaqMan® Human Endogenous Control Plate that enables the evaluation of 11 endogenous control genes. HEK293 cells were cultured for 14 days in conditions of depleting folate. Decline in cellular folate levels was confirmed by intracellular folate assay. Duplicate cDNA samples from Day0, 3, 8 and 14, representing different degrees of folate depletion, were used. RT-PCR was performed on an ABI 7500 PCR System. GUS control gene was shown to be the endogenous control gene that displayed the least amount of variation across samples and therefore is the most accurate choice as a single normalisation gene for HEK-293 cells under conditions of depleting folate.

P25. Down syndrome and Achondroplasia: A rare combination

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Achondroplasia and Down syndrome are the commonest genetic conditions within their respective categories. Presence of these two genetic entities in the same patient is a rare event and has been reported only thrice in medical literature. We report the fourth case with this rare combination.

The proband was a female infant born at term to a Caucasian couple with maternal and paternal age of 41 and 43 years respectively. The clinical features included frontal bossing, flat nasal bridge, down slanting palpebral fissures, long philtrum, thin lips and bilateral simian creases and Tetralogy of Fallot. The clinical diagnosis of Down syndrome was confirmed by karyotyping. She also had relatively large head, depressed nasal bridge, rhizomelic shortening of all limbs, protuberant abdomen and trident configuration of both hands. These features were suggestive of achondroplasia and the radiological features were consistent with this diagnostic possibility. FGFR3 gene mutation analysis showed G380R G > A mutation.

The combination of Down syndrome and achondroplasia in our patient is likely to be a chance event because of the advanced parental ages. Molecular confirmation of achondroplasia is not routinely requested. However it was extremely useful in this case from diagnosis and counselling point of view.

P26. Natural history of Williams Syndrome: a report of 2 cases

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Williams syndrome (WS) is a well described genetic syndrome affecting 1 in 20,000. However there is relative paucity of medical literature devoted to adults with this condition. We report 2 adults with WS diagnosed in their late 50s.

Case 1: 57 year old lady referred with clinical suspicion of Turner syndrome. She has learning difficulties, short stature, kyphoscoliosis, joint stiffness, cardiac pacemaker for complete heart block, abnormal glucose tolerance test, hypertension and constipation. She lives in a residential home with her older sister who also has learning difficulties and short stature.

Case 2: 57 year old man referred to genetics department with clinical features, mild learning difficulties, diabetes, sensorineural hearing loss, constipation, and was operated for inguinal hernia, bladder diverticulae, aortic valve replacement and aneurysm of ascending aorta. He also had stroke and has never lived independently.

These are probably the oldest reported cases of WS. Their clinical features and the associated medical complications delineate the natural history of this condition. It also highlights the need for better understanding/ awareness of this condition among professionals working in adult services.

P27. Implementation of a Luminex-based CF Assay at NCMG – A Validation Experience

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With the aim of improving the efficiency of the NCMG cystic fibrosis (CF) service, we looked for a multiplexed CF assay which could be adapted to the mutation spectrum of the Irish population.

Using Luminex™ Liquid Bead Array Platform (Applied Cytometry), we evaluated the Signature™ CF 2.0 ASR from Asuragen which tests for 25 of the CF mutations included in the ACMG/ACOG recommended CF panel.

We evaluated this assay on a variety of sample types and on a large cohort (n=468) of DNA samples of known genotypes to examine sensitivity and specificity. All samples except one were genotyped correctly during this initial validation, indicating that the Signature™ CF 2.0 ASR was a sensitive and robust assay for CF diagnostics. We observed a discordant result between our ARMS assay and the Signature™ CF 2.0 ASR for one sample. Subsequent investigations revealed this discrepancy to be due to the presence of CF mutation V520F, which resulted in non-amplification of the mutant allele due to its position under the exon 10 forward primer in the Signature™ CF 2.0 ASR.

We describe collaborative efforts by NCMG and Asuragen to address the issue of SNPs under primers in commercial ASRs.

P28. Partial trisomy 13: A case report, verification of the phenotype and review of the literature

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Partial trisomy for distal chromosome13 has previously been described in 20 patients. The reported phenotype consists of microcephaly, distinctive facies, high arched palate, postaxial polydactyly, genitourinary anomalies, 2/3 toe syndactyly, moderate mental retardation and relatively few major malformations.

We describe a further case in a male infant born by emergency C/S to a primagravida due to failed induction at T+3, after an uneventful antenatal history. Birth weight was on 75th centile. On examination he presented with striking dysmorphic features, a high arched palate, long fingers and toes with bilateral postaxial polydactyly, bilateral 2/3 soft tissue toe syndactyly and hypospadias.

Cytogenetic analysis revealed additional chromosome material at 9p24.1 which was confirmed as 13q22.3->qter by FISH studies. Subsequent parental chromosome analysis indicated that the unbalanced rearrangement had arisen from an adjacent I segregation of a maternal t(9;13)(p24.1;q22.3).

This case provides further evidence that trisomy 13q22.3->qter presents with a characteristic spectrum of abnormalities. A review of the literature indicates that, of the features of full trisomy 13, congenital heart defects, clinodactyly and frontal bossing appear to be associated with proximal 13q trisomy, while genitourinary anomalies, micropthalmia, cleft palate and polydactyly are more prevalent in trisomy for distal 13q.

P29. A Genotype-Phenotype Correlation Study In An Extended Irish Kindred With Variegate Porphyria Caused By *PPOX* Q435X

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Variegate porphyria (VP) is genetically heterogeneous, and demonstrates variable penetrance and expressivity of clinical and biochemical phenotype within affected families. Phenotypic variability may be related to the nature of the underlying pathogenic mutation. Q435X is the most common VP-causing mutation encountered in the UK (7%) and we report a genotype-phenotype correlation study of Q435X in an extended Irish kindred

Molecular genetic scanning of *PPOX* identified a nonsense mutation Q435X in two subjects with confirmed VP. A further twenty-two adult members of this extended family were screened for Q435X. In total 67% (16 out of 22) were mutation positive. Plasma fluorescent emission spectroscopy (PFS) screening was also undertaken in all subjects, and had a specificity of 100% but sensitivity of only 80%. A clinical questionnaire revealed that only 19% (3 out of 16) of mutation positive subjects had clinically overt cutaneous manifestations of VP and13% (2 out of 16) had experienced acute episodes.

The results of this genotype-phenotype study suggests that Q435X demonstrates a less penetrant cutaneous phenotype but greater penetrance of acute neurovisceral attacks than a well characterised South African founder mutation R59W. Furthermore, PFS was only 80% sensitive, thus confirming that mutation analysis is diagnostically superior in the detection of presymptomatic carrier status.

P30. The Molecular Basis of Acute Porphyria in the Republic of Ireland.

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Acute porphyrias, which include acute intermittent porphyria (AIP), variegate porphyria (VP) and hereditary coproporphyria (HP), are autosomal dominantly inherited disorders affecting key enzymes in the haem biosynthetic pathway, and demonstrate variable penetrance (20%) and expressivity. Clinically these disorders may manifest with photosensitive skin lesions (VP and HP) and/or acute neurovisceral episodes (AIP, VP and HP), the latter being potentially associated with significant morbidity. While biochemical investigations, including blood, urine and faecal porphyrin analysis, are critical for the diagnosis of active porphyric disease, these investigations may not be sensitive enough to identify presymptomatic mutation carriers. Hence molecular genetic analysis has become an important component in kindred follow-up for identifying porphyria susceptibility.

The Biochemistry Department, St James's Hospital, Dublin, in collaboration with Cardiff Porphyria Centre, have recently established a biochemical genetic service for the acute porphyrias. Mutation scanning using PCR and direct nucleotide sequencing has identified 11 different mutations in 12 porphyria kindred within the Republic of Ireland. This includes mutations in *HMBS* (R26C, R26H, IVS4+1G>A), *PPOX* (IVS4-1G>A, Q435X, W427X, A150D, Q375X) and *CPO* (R332Q, R332W, c.1291-1292 ins TG), causing AIP, VP and HP respectively.

This unique insight into the molecular basis of porphyria in the ROI population clearly indicates that acute porphyrias are genetically heterogenous within this cohort.

P31. Genetic variants of Complement factor H gene are not associated with premature coronary heart disease: a family-based study in the Irish population

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Background: The complement factor H (CFH) gene has been recently confirmed to play an essential role in the development of age-related macular degeneration (AMD). There are conflicting reports of its role in coronary heart disease. This study was designed to investigate if, using a family-based approach, there was an association between genetic variants of the CFH gene and risk of early-onset coronary heart disease.

Methods: We evaluated 6 SNPs and 5 common haplotypes in the CFH gene amongst 1494 individuals in 580 Irish families with at least one member prematurely affected with coronary heart disease. Genotypes were determined by multiplex SNaPshot technology.

Results: Using the TDT/S-TDT test, we did not find an association between any of the individual SNPs or any of the 5 haplotypes and early-onset coronary heart disease.

Conclusion: In this family-based study, we found no association between the CFH gene and early-onset coronary heart disease.

P32. Identifying potential candidate genes in an Irish bipolar disorder sample linked to 14q21-32.

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Bipolar affective disorder (BPAD) is a severe and debilitating psychiatric illness. Family, twin and adoption studies have established a substantial genetic component to the illness but the genes involved have yet to be fully elucidated. A 10cM genome-wide linkage scan (WGS) was performed in a collection of 60 Irish BPAD affected sib pairs to locate chromosomal regions that may harbour susceptibility genes. The most significant result was on chromosome 14 at 75cM (14q24). Since the region of the chromosome containing significant *P* values was substantial, we undertook a fine-mapping analysis to refine the linkage peak. 144 SNP markers (400kb resolution) were analysed in an extended sample of 88 ASPs. Linkage analysis resolved our original linkage peak into 4 separate peaks, two of which overlap with published linkage peaks for related psychiatric disorders, such as anxiety and alcoholism. The most significant NPL score of 2.71 was at 67.84Mb, remarkably close to the original WGS peak score at 68.2Mb. In an additional analysis, two SNPs were found to be associated with BPAD (rs24166076 at 46.97Mb and rs4902942 at 71.21Mb). This project has substantially refined the region of chromosome 14 predicted to contain a candidate susceptibility gene for BPAD.

P33. Estimating carrier risks by linkage in a Duchenne Muscular Dystrophy family with a triple X female

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We received a referral for carrier status on a female with a brother deceased with a clinical diagnosis of DMD, and no genetic testing done. There was no other family history of DMD. The consultand's mother had a CK of 480, and a number of other children (2 daughters and 1 unaffected son), all of whom had different fathers. The two eldest daughters had been adopted separately at an early age, and were also requesting information regarding carrier status. DNA samples from their fathers were unavailable, but we did receive a sample from their unaffected half-brother. Thus, the request was for linkage analysis in a very unusual and complicated pedigree, where samples were unavailable from many significant family members, including the index case. Linkage analysis commenced, yielding unexpected results which provided evidence of 3 distinct alleles at 5', intergenic, and 3' Dystrophin polymorphic markers in the index case's mother. Subsequent cytogenetic analysis confirmed a 47, XXX karyotype. Despite the presence of three Dystrophin haplotypes in her, and the complex pedigree, we were successful in haplotyping the family. Furthermore, it was possible to assign carrier risks to all at-risk females, two of whom were estimated to be at a <1% risk.

P34. Distal Duplication 10q: a case of gonadal mosaicism?

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Partial duplication of chromosome 10q is a rare abnormality usually associated with significant dysmorphism and intellectual deficit. The majority of published reports relate to segments encompassing up to one third of the long arm. Typically another chromosome is involved, which is likely to influence the phenotype. We present two sibs with a pure duplication of the most distal bands of chromosome 10q who show a relatively mild phenotype. TM and EM are sisters, in a sibship of five, who present with developmental delay but no significant dysmorphic features. Cytogenetic analysis demonstrated a small distal duplication comprising bands 10q26.13 to 10q26.3. Parental karyotypes were normal, suggesting gonadal mosaicism. Phenotypic expression is more developed in TM, the elder sister, who is now 10 years old. She presents with learning and behavioural difficulties with minor stereotypic movements, low muscle tone, hyperextensible joints, slight bilateral clinodactyly and exaggerated lumbar lordosis. The mild phenotype is clearly a function of the small size of 10q duplication in contrast to the severe phenotype normally observed.

